Infection Studies of *Diplodia zeae* (Schw.) Lev. and Control of Seedling Blights of Corn

By W. P. Raleigh

AGRICULTURAL EXPERIMENT STATION
IOWA STATE COLLEGE OF AGRICULTURE
AND MECHANIC ARTS

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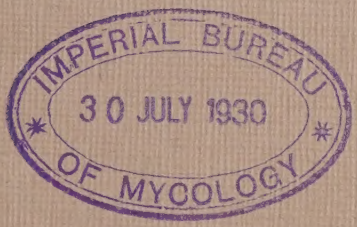
BOTANY SECTION



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SUMMARY

The two most important symptoms of the corn disease caused by *Diplodia zeae* are seedling blight and ear rot.

Artificial inoculation of the ears was effected in all stages of their development but most readily in the milk stage.

Infection of seedlings from Diplodia-infected kernels usually takes place in the natural wounds at the points of production of the secondary radicles near the attachment of the mesocotyl to the scutellum.

Field experiments showed that Diplodia-infected seed in 1925 and 1927 produced 4.5 and 1.3 times as many weak plants, respectively, as did nearly disease-free seed.

Plants that are weak due to *Diplodia zeae* and *Basisporium gallarum* early in the season often produce normal ears if there is no competition in the hill but seldom produce normal ears if there is competition with strong plants.

The visible-root sand culture method, devised as a means of following the spread of Diplodia in the underground parts of the corn seedling, has been found of value in correlating micro-organism injury or disinfectant injury with top growth and in determining the effect of moisture and temperature on the development of Diplodia seedling blight.

Fewer plants developed from Diplodia-infected seed at 15°-19° C. than at 20°-24° C. There was less visible seedling blight at the lower temperature, due to the large number of seedlings that were killed before they reached the surface of the soil.

Results of seed treatment experiments in 1926 using nine mercury disinfectants on nearly disease-free seed, showed that yields were not increased or decreased significantly. However, in 1927, treated, high quality, selected seed corn entered in the state yield contest gave significant increases. In 1926, Diplodia-infected treated seed showed significant increases ranging from 6.8 to 13.9 bushels per acre. The yield from the highest yielding, Diplodia-infected, treated seed was within about six bushels of that from the nearly disease-free seed. In 1927, three mercury dust disinfectants (Bayer Dust, Merko and Semesan Jr.) increased the yield of Diplodia-infected seed from 21.3 to 30.2 percent and of Basisporium-infected seed from 15.5 to 19.6 percent.

The benefits derived from treatment of seed corn vary greatly with the amount of infection in the seed, with the climatic and soil conditions at planting time and apparently with the strain of corn used.

Infection Studies of *Diplodia zeae* (Schw.) Lev. and Control of Seedling Blights of Corn.*

By W. P. Raleigh

Altho the organism, *Diplodia zeae* (Schw.) Lev. was first described over a century ago, it was not recognized as the cause of a serious disease of corn until Heald (4) published his first note on Diplodia ear rot in 1906. During the next few years Heald, Wilcox and Pool (5), and Burrill and Barrett (1) contributed very substantially to the ear rot phase of the problem, but they did not deal with the relation of this disease to the seed. This last phase of the problem was not studied until the beginning of the present decade. It was Durrell (3), and Holbert, Burlison et al. (6) who directed themselves to the infected seed phase and showed that *D. zeae* is often responsible for serious reduction in stand and yield.

Seed treatment studies were initiated in several laboratories soon after it was established that Diplodia was a seed-borne disease which was often responsible for unsatisfactory field stands and yields. These later studies showed rather conclusively that more detailed data on infection, especially of the kernels and seedlings, were necessary before the problem of control thru seed treatment could be properly attacked.

This publication deals mainly with infection of the ears and kernels with *Diplodia zeae,* the development of the seed-borne organism as the cause of a seedling blight and the control of this and other seedling blights of corn thru seed treatment. Special emphasis has been placed on factors influencing the progress of the Diplodia organism from the seed to the seedling and on laboratory methods of testing disinfectants with regard to their ability to check this progress and thereby prevent seedling blight.

Natural Infection of Ears.

The matter of infection of the ears in the field with *Diplodia zeae* has received attention for some time. Burrill and Barrett (1), using glass plates coated with a mixture of glycerin and alcohol, demonstrated that it was possible for Diplodia spores to be blown 350 yards from old stalks. Durrell (3) showed that

*The work reported in this bulletin has been done in the Plant Pathological Laboratory of the Iowa Agricultural Experiment Station under Furnell Project No. 32 on Corn Diseases, under the direction and supervision of Dr. I. E. Melhus, experiment station plant pathologist.

weather conditions had a marked influence on the amount of Diplodia-infected ears. He found that those sections of the state of Iowa having a large amount of rainfall during August and September, 1921 and 1922, almost invariably had a higher percentage of Diplodia-infected ears than those sections having less rainfall.

The question of whether or not the planting of Diplodia-infected seed results in a higher percentage of Diplodia-infected ears has received some attention. Smith and Hedges (12) suggest systemic invasion of the plants, but later work of Durrell (3) showed that infection of the ears did not take place in this manner. Durrell (3) and Clayton (2) found that Diplodia earrot occurred as frequently on plants from disease-free seed as on plants from Diplodia-infected seed.

Data along these lines were obtained at Iowa State College in 1926 by counting the number of Diplodia-rotted ears in two adjoining seed treatment plots of corn, each consisting of 101 rows, 30 hills long. One was planted with Diplodia-infected and the other with nearly disease-free seed. The data showed that 3.3 percent of the 5,821 ears from the nearly disease-free seed were either partially or wholly overrun with *Diplodia zeae,* and 6.5 percent of the 4,921 ears from the adjoining plot from Diplodia-infected seed were either partially or wholly over-run by the organism. As these plots were side by side in the same field the amount of inoculum or refuse in each, from the preceding year should have been practically the same and, therefore, probably had no relation to the difference in infection. The Diplodia-infected and nearly disease-free seed were selected from the same lot of corn. Whether or not a similar response would occur another season is not known, but the difference in percentages of infection is statistically significant. A probable explanation is that the fungus grew from some of the infected kernels, fruited just above the crowns on the lower dead leaf sheaths of the plants and produced additional inoculum. Still another possible explanation of the difference in the amount of ear infection is that some of the plants from Diplodia-infected seed were retarded so that the ears were less mature and as a result were more susceptible at the time when weather conditions for infection with *D. zeae* were most favorable. Regardless of the explanation, these data are not in agreement with previous similar data.

Artificial Infection of Ears in the Greenhouse and Field.

Two inoculation experiments with *Diplodia zeae* were conducted in the greenhouse in the fall of 1926. In one experiment, in which dent corn was used, infection was produced on ears in the blister stage of development by placing the spores on the outside of the husks and maintaining a high moisture condition about

the ears by inclosing them in celluloid cylinders plugged at each end with cotton. The temperature during the inoculation period ranged from 25° to 40° C.

In another experiment, sweet corn ears, var. Golden Bantam, were used with the husks retained and at two stages of development. Fifty healthy ears in the milk stage were placed on end in four-gallon earthen jars with a diseased ear placed near the center in each jar. The same procedure was followed using 50 healthy ears and one diseased ear in the dent stage. At the end of 10 days the ears in the milk stage, that were in contact with the diseased ear, were badly overrun with *Diplodia zeae*, but the ears in the dent stage similarly placed were not noticeably infected.

In order to learn more about natural infections of *Diplodia zeae*, field experiments were conducted in 1927 on the station plots, in which ears were inoculated at different times and by diffent methods, and the subsequent development of Diplodia ear rot was noted.

In all inoculations (unless otherwise specified) a culture of *Diplodea zeae* isolated from an old corn stalk was used. This culture produced abundant pycnidia when grown on sterilized wheat. The possibility of strains of the organism was not investigated.

Each week from Aug. 22 to Sept. 20, from 50 to 100 ears of dent corn were inoculated in the field as follows:

(1) Approximately 5 cc. of a spore suspension of *Diplodia zeae* was poured on the silks of each ear.

(2) In other trials about 5 cc. of a spore suspension of *Diplodia zeae* was poured on the shank of each ear, under the leaf sheath.

(3) A Diplodia-infected wheat kernel was inserted in the shank of each ear about one-half inch from the point of its attachment.

Fifty ears from each group were harvested Sept. 28 and, supported on wire frames, were allowed to dry rapidly in the seed house. Twenty-three days after harvesting a germination test of these ears was made. Ten kernels equi-distant from each other were taken from what appeared to be an average row on each ear. These were placed in modified rag dolls in the order of their position on the ear. Conditions were made favorable for the germination of the corn and the growth of Diplodia. At the end of seven days, records of infection were taken. The remaining ears (from 20 to 50) were harvested Nov. 17 and tested on Jan. 21 in the same manner. The results are recorded in table I.

Table I shows that infection was obtained by all three methods of inoculation. The method producing the highest percentage of infected ears was that in which an infected kernel of wheat was inserted in the shank of each ear. By this method the inoculum

TABLE I. DATA OBTAINED FROM GERMINATION TESTS OF EARS OF YELLOW DENT CORN INOCULATED IN THE FIELD AT DIFFERENT TIMES AND BY THREE DIFFERENT METHODS WITH *DIPLODIA ZEAE*.

Date of inoculation	Method of inoculation	Stage of development	No. ears inoculated	Date harvested	Date of germination test	Percentage of ears infected	Per-cent'ge of infected kernels on infected ears	Per-cent'ge dead kernels on infected ears
Aug. 22	Inoculum inserted in shank	Blister	50	Sept. 28	Oct. 21	94	96.4	93.4
Aug. 30	" " "	Early milk	50	Sept. 28	Oct. 21	100	91.6	86.6
Sept. 6	" " "	Milk	50	Sept. 28	Oct. 21	98	73.5	50.8
Sept. 12	" " "	Late milk	50	Sept. 28	Oct. 21	78	49.3	19.7
Sept. 12	" " "	Late milk	20	Nov. 17	Jan. 21	95	68.9	49.5
Sept. 20	" " "	Early dent	50	Sept. 28	Oct. 21	0	0	0
Sept. 20	" " "	Early dent	20	Nov. 17	Jan. 21	40	45.0	13.8
Oct. 4	" " "	Dent	20	Nov. 17	Jan. 21	15	30.0	30.0
Aug. 22	Spores placed on shank	Blister	50	Sept. 28	Oct. 21	24	32.0	21.9
Aug. 30	" " "	Early milk	50	Sept. 28	Oct. 21	30	66.7	37.3
Sept. 6	" " "	Milk	50	Sept. 28	Oct. 21	0	0	0
Sept. 12	" " "	Late milk	50	Sept. 28	Oct. 21	0	0	0
Sept. 12	" " "	Late milk	20	Nov. 17	Jan. 21	15	43.3	40.0
Sept. 20	" " "	Early dent	50	Sept. 28	Oct. 21	4	30.0	0
Sept. 20	" " "	Early dent	20	Nov. 17	Jan. 21	10	40.0	15.0
Oct. 4	" " "	Dent	20	Nov. 17	Jan. 21	0	0	0
Aug. 22	Spores placed on silks	Blister	50	Sept. 28	Oct. 21	72	99.7	87.7
Aug. 30	" " "	Early milk	50	Sept. 28	Oct. 21	66	93.0	68.2
Sept. 6	" " "	Milk	50	Sept. 28	Oct. 21	18	60.0	32.2
Sept. 12	" " "	Late milk	50	Sept. 28	Oct. 21	18	41.1	15.5
Sept. 12	" " "	Late milk	20	Nov. 17	Jan. 21	35	70.0	61.4
Sept. 20	" " "	Early dent	50	Sept. 28	Oct. 21	0	0	0
Sept. 20	" " "	Early dent	20	Nov. 17	Jan. 21	10	55.0	15.0
Oct. 4	" " "	Dent	20	Nov. 17	Jan. 21	0	0	0

was inserted in the moist shank of the ear where it was protected from drying. The lowest percentage of infected ears was obtained by pouring the spore suspension of *Diplodia zeae* on the shanks of the ears, and a moderately high percentage of infected ears was obtained where the spore suspension was poured on the silks.

In general the early inoculations produced much higher percentages of infection than late inoculations. Also, the early inoculations usually produced high percentages of infected kernels. Less than 3 percent of Diplodia-infected ears occurred in the checks.

The results are in accord with those of Burrill and Barrett (1) and Clayton (2) in that higher percentages of infected ears were obtained by inoculating when the corn was in the milk stage when it was nearer maturity. The results are also in accord with the work of Durrell (3) in which he found that Diplodia infection under natural conditions was greatly influenced by moisture relations.

The fact that a higher percentage of infected ears was obtained by silk than by shank inoculations without wounding is not in accord with what occurs in nature, or to field observa-

tions during these investigations, or with Durrell (3), Holbert, Burleson et al. (6) and Clayton (2), because under natural conditions more ears are infected with *Diplodia zeae* at the base than at the tip. Possibly this difference is due to the fact that the spores poured on the silks were placed well down between them rather than merely in the tip as would occur in nature. It is evident that under natural conditions the spores that come to rest in the silks would often be pushed some distance from the ear before they would have time to germinate. Especially is this true during the time of rapid growth of the silks.

Results bearing on the practice of early selection of seed corn were obtained from the inoculations made Sept. 20. None of the ears harvested Sept. 28 were infected by *Diplodia zeae*, but 40 percent of the ears harvested later, Nov. 17, were infected.

Spread of Diplodia zeae in Curing and Storage of Seed Corn.

If the organism, *Diplodia zeae*, is present in seed ears when they are selected, it seems to continue to grow until checked by unfavorable conditions of moisture or temperature. It is evident, therefore, that curing and storage conditions often play an important part in the production of good and bad seed corn.

An experiment was conducted in 1927 to determine the effect of curing and storing Diplodia-infected yellow dent corn ears under moderately good conditions and poor conditions. Sixty ears infected at the tip and 100 ears infected at the butt were harvested Sept. 28, and half of each was placed under the two kinds of storage conditions. Data on the germination and infection of each ear were determined on the germinator on Oct. 21 and again on Jan. 21. Ten kernels were used each time an ear was tested. A summary of these data is presented in table II.

Table II shows that the percentage of kernels infected with *Diplodia zeae* was in all cases greater in the late (winter) than in the early (fall) test, indicating that there was progress of the organism during the curing and storage period. The data also show that under poor storage conditions there was a large increase in the percentage of dead kernels in the winter as compared to the fall tests. This again indicated that there had been

TABLE II. PROGRESS OF *DIPLODIA ZEAE* IN INFECTED EARS CURED AND STORED UNDER MODERATELY GOOD AND POOR CONDITIONS AS SHOWN BY TWO GERMINATION TESTS OF 10 KERNELS EACH FROM 60 EARS INFECTED AT THE TIP AND 100 EARS INFECTED AT THE BASE.

Location of infection	Kind of storage	23 Days in storage			115 Days in storage		
		Percent not infected	Percent infected		Percent not infected	Percent infected	
			Alive	Dead		Alive	Dead
Tip	Mod. good	44.33	21.67	34.0	29.0	42.33	28.67
Tip	Poor	29.67	27.67	42.67	13.0	30.0	57.0
Base	Mod. good	35.2	22.2	42.6	23.8	39.6	36.6
Base	Poor	33.8	23.0	43.2	21.2	29.4	59.4

considerable growth in storage. In the case of the ears cured and stored under moderately good conditions, the small decrease in the percentage of dead kernels may have been due to slight variations in the conditions surrounding the germination trials.

Kernel and Seedling Infection.

There is naturally a very close relationship between ear, kernel and seedling infection. Just how the organism invades the kernel and how it spreads in the tissues, as well as how it makes its way from the germinating kernel into the tissues of the mesocotyl, is not well understood. In the same way the influence of moisture and temperature on the migration of the organism needs consideration. The data accumulated on these phases of the problem comprise observations of germination tests of hundreds of ears infected with *Diplodia zeae,* histological studies and laboratory and field tests.

As was previously stated, primary infection of the ears by *Diplodia zeae* is usually at the butt and less often at the tip. The kernels on such ears may show different degrees of infection: (1) a zone of dead kernels around the point where the pathogene entered, (2) another zone in which the kernels are infected but still germinate and (3) a zone in which the kernels are entirely free from infection. Therefore, an ear attacked by *D. zeae,* unless entirely overrun by the pathogene, shows different degrees of infection. Even within the zone of infected kernels that grow, there are varying degrees of fungous invasion (fig. 1). In

Fig. 1. Corn kernels after six days on the germinator showing the effect of different degrees of infection with *Diplodia zeae.* Kernel No. 1 was severely infected and killed before germination; the seedling from kernel No. 2 was killed when the radicle was about 2 inches long and the plumule had just burst the pericarp; the seedling from kernel No. 3 was badly stunted due to an infection near the vascular zone; and the mesocotyl from kernel No. 4 was slightly invaded as indicated by the small lesion beginning in the wounded tissue caused by the emergence of the secondary radicles.

Fig. 2. Primary infection with *Diplodia zeae* is shown by the small lesion around the natural wound at the base of one of the secondary radicles. This seedling was grown on the germinator from a slightly infected corn kernel.

Fig. 3. Diplodia-infected corn seedling sectioned so as to show the lesion in the region where the secondary radicles emerged.

stained sections it is possible to find strands of the mycelium immediately under the tip cap of slightly infected kernels. This, together with observations on the germinator, suggests that the organism lies in the region of the tip cap. In slightly infected kernels, placed under conditions for germination, both the organism and the kernel grow. On the germinator, heavily infected kernels often fail to germinate or are killed soon after they germinate. When the kernels are slightly infected, the seedlings may make considerable growth before they are injured by the organism. Observations of slight and medium infected kernels on the germinator show that infection of the seedling usually begins at the points where the secondary radicles emerge. The secondary radicles emerge from the lower part of the mesocotyl just before it terminates at the vascular zone or point of attachment to the scutellum. The organism apparently enters the natural wounds made by the emergence of the secondary radicles (figs. 1, 2 and 3). After penetration, the organism is usually restricted to the mesocotyl, which seems to be the most susceptible tissue of the corn seedling.

Observations during 1927 point to the fact that it is probable that under certain conditions the organism may progress from the underground part of the seedling up the plant to the surface of the soil. On Aug. 15 Diplodia pycnidia were found on the lower portions of sweet corn plants, var. Golden Bantam (fig. 2). The infected plants were dying while healthy plants were in the milk stage. In nearly all cases, individual stalks and not whole hills were infected, which suggested the probability of infection from the seed and not from inoculum in the soil.

The viability of the spores produced on these sweet corn plants was tested in the following experiment: Eighteen ears of yellow dent corn were inoculated by inserting the spores in the shank of

each ear on Aug. 30, 1927. The ears were harvested Nov. 17, when it was found that five of the eighteen ears were either partially or wholly overrun by Diplodia mycelium. Unmoculated ears held as checks showed less than 3 percent infection.

Field Data on Weak Plants.

Under field conditions, as has been noted, the planting of Diplodia-infected seed may result in a poor stand especially

Fig. 4. Two stalks of sweet corn, var. Golden Bantam, infected by *Diplodia zeae*. Note the blackening of the lower portion of the stalk and the abundance of pycnidia in and above the discolored area. These plants were dead when apparently normal plants in the field had ears in the late milk stage.

TABLE III. PERCENTAGES OF WEAK PLANTS IN FIELD PLOTS PLANTED WITH DIPLODIA-INFECTED SEED AND WITH NEARLY DISEASE-FREE SEED.

Date	Kind of seed planted	Av. height plants in inches	No. plants	No. weak plants	Percentage weak plants
1925	Nearly disease-free	4	3135	56	1.8
1925	Diplodia-infected	4	1646	133	8.1
1927	Nearly disease-free	36	1099	58	5.3
1927	Diplodia-infected	36	776	55	7.1

when the soil is cool and moderately wet. It has also been noted that Diplodia-infected seed produces more weak plants than does nearly disease-free seed. In order to obtain additional evidence on this point, data on the number of weak plants were recorded from experiments in 1925 and 1927 on the station plots. In 1925 the determinations were made when the plants were about 4 inches in height. Plants were recorded as weak if they were less than two-thirds of average height. The same method was used in 1927, but the records were taken when the plants averaged about 3 feet in height. The results of these studies are shown in table III.

Data in table III show that in both years there was a larger percentage of weak plants in the plot from Diplodia-infected seed than in the plot from nearly disease-free seed. The difference in percentage of weak plants was greater in the 1925 (statistically significant) than in the 1927 (not statistically significant) plots, probably because the data were taken at an earlier stage of plant development in 1925 than in 1927.

The field performance of weak corn plants was determined in an experiment conducted in 1925. However, in this experiment the weak plants resulted both from seed infected with *Diplodia zeae* and with *Basisporium gallarum*. Therefore, the results cannot be ascribed definitely to the Diplodia dry rot disease. The weak plants were marked when the average height in the field was about 4 inches. Records were taken at harvest time. The term "nubbins" is used to designate ears less than 4 inches long,

TABLE IV. INDIVIDUAL PERFORMANCE OF WEAK CORN PLANTS GROWING SINGLY AND IN ASSOCIATION WITH STRONG AND WEAK PLANTS, OR WITH BOTH, USING THE HILL AS A UNIT, THE PLOTS BEING PLANTED THREE KERNELS PER HILL.

Kind and number of plants in hill	No. hills	Percent normal ears	Percent small ears	Percent nubbins	Percent barren stalks
1 weak	53	85	5.5	7.5	2
1 weak and 1 strong	45	44.5	20.0	24.5	11
1 weak and 2 strong	32	22	6	44	28
2 weak	50	73	7	12	8
2 weak and 1 strong	11	41	0	32	27
3 weak	24	48	23	19	10

and normal ears are ears over 6 inches. A summary of the data is given in table IV.

The data in table IV show that in general the yields from weak plants differed principally in relation to the amount of competition in the hill. Eighty-five percent of the weak plants when growing singly in a hill produced normal ears. When a weak plant was in association with one strong plant in a hill, normal ears were produced in 44.5 percent of the cases. In association with two strong plants in a hill, only 22 percent of the weak plants produced normal ears. When the hill consisted of two weak plants only, normal ears were produced in 73 percent of the cases; while, when two weak plants were in association with one strong plant, only 41 percent of the weak plants produced normal ears. When three weak plants were together in a hill, they produced normal ears in 48 percent of the cases. In all cases, where the percentage of normal ears was low, the percentage of barren stalks and nubbins produced was high and vice versa. Again in 1927, the field performance of weak plants was determined in an experiment using Diplodia-infected seed planted two kernels per hill. In this experiment, the plants were marked in July when the average height was about 3 feet. Records were taken at harvest time and a summary of the data is given in table V.

TABLE V. INDIVIDUAL PERFORMANCE OF WEAK CORN PLANTS GROWING SINGLY AND IN ASSOCIATION WITH ONE STRONG PLANT, USING THE HILL AS A UNIT AND PLANTING TWO KERNELS PER HILL.

Kind and number of plants in hill	No. hills	Percent normal ears	Percent small ears	Percent nubbins	Percent barren stalks
1 weak	34	14.7	29.4	20.6	35.3
1 weak and 1 strong	45	2.2	4.4	26.7	66.7

The data in table V are in accordance with the results in 1925 in that fewer good ears were produced and more barren stalks resulted when there was competition with a strong plant in the hill. The fact that weak plants produced more poor ears or resulted in more barren stalks in 1927 than in 1925 may be accounted for in part by the delay in marking until the plants were much further advanced. Results indicate that plants that are weak as late in the season as July have little chance to recover fully, especially during a dry season such as that of 1927.

The Visible Root Sand Culture Method.

In this study of seedling infection, it became necessary to devise ways and means of following the spread of Diplodia in the underground parts of the seedling. Reddy and Holbert (10) used a somewhat elaborate method consisting of soil cans 8 inches

in diameter and 10 inches deep. They placed 6 inches of moistened soil in the bottom of the cans, then added a 3-inch layer of sand, and planted the corn at a depth of 1 inch. After trying this method and many others that were improvised, it was found that planting corn in crystallizing dishes covered with 4 centimeters of sand proved very convenient for following continuously the development of Diplodia on the underground parts of the corn seedling.

The use of crystallizing dishes with sand as described in these studies will be known hereafter as the visible root sand culture method. A detailed description of the method follows. Crystallizing dishes 10 centimeters in diameter and 5 centimeters deep were used as containers. From 15 to 25 kernels of corn were evenly distributed in the bottom of each dish. River sand was then added to a depth of 4 centimeters. An equal amount of water was added to each culture at the beginning of the test and later if necessary.

This method of culture proved especially adaptable for studying the rate of spread of infection in the underground parts of corn seedlings, for testing the comparative value of seed corn treatments, for following the effect of moisture and temperature on the development of Diplodia and for testing the relative resistance of different varieties and strains of corn.

Temperature and Moisture in Relation to Seedling Blight.

In order to test the effect of temperature and moisture on the development of Diplodia seedling blight from seed corn infected with *Diplodia zeae*, a laboratory trial was conducted, using naturally infected seed that showed over 65 percent Diplodia-infected kernels in a germinator test. Using the visible root sand culture method, 25 kernels were placed in the bottom of each of 48 dishes, which were divided into three groups of 16 each. An equal amount of river sand of a known moisture content was added to each dish in two of these groups, and enough water was added to bring the sand to the desired moisture content, namely, 95 and 60 percent of their water holding capacity, respectively. To each one of the third group of dishes, the same amount of sand was added after it was adjusted to 25 percent of its moisture holding capacity. In the latter, the water was added to the sand before it was placed in the dishes because this small amount of water would not distribute itself readily when added to the dry sand in the dishes. After these sand cultures were adjusted to their respective moisture contents, each group of 16 was divided as follows: eight were placed in the laboratory at a temperature of 20°-24° C. and the other half in a greenhouse at a temperature of 15°-19° C. Each day the cultures were weighed and water added to bring them to their original weight. After a two-week

TABLE VI. DATA ON THE EFFECT OF TEMPERATURE AND MOISTURE ON THE DEVELOPMENT OF SEEDLING BLIGHT IN PLANTS FROM SEED CORN NATURALLY INFECTED WITH *DIPLODIA ZEAE*, EACH DETERMINATION BEING MADE WITH 200 KERNELS PLANTED 25 KERNELS IN EACH OF 8 DISHES, USING SAND AS A MEDIUM.

Temperature (degrees C.)	Percentage of moisture holding capacity	Percent kernels not germinating	Percent seedlings not emerging	Percent seedlings emerged	Percent seedlings free from lesions	Weight of green tops in grams
20-24	95	20.5	22.5	57.0	0.5	11.5
20-24	60	13.5	30.0	56.5	2.5	9.0
20-24	25	19.0	10.0	71.0	2.0	5.0
15-19	95	10.0	49.0	41.0	8.0	8.75
15-19	60	12.0	54.0	34.0	7.5	5.75
15-19	25	15.0	46.5	38.5	12.0	3.9

period, the plants were washed free from the sand. Data on germination of the seed and infection by *D. zeae* are presented in table VI.

From the results recorded in table VI, an important effect of temperature on Diplodia seedling blight may be noted. Altho just as many kernels germinated at the low temperature, the stand or number of plants emerged was greater at the higher temperature. On examination, it was found that many of the infected plants at the lower temperature were killed before they reached the surface. More plants showed lesions, and the lesions were more extensive at the higher than at the lower temperature.

These results are in accord with field experiments at Ames and with those of Holbert, Burlison, et al. (6), in that better stands were obtained from Diplodia-infected seed in warm than in cold soils. They are not in accord with the conclusions of Durrell (3) and Clayton (2) in their statements that seedling blight from seed infected with *Diplodia zeae* is not important at the relatively cool temperatures prevalent in the spring. Both of these investigators obtained poor stands at low temperatures, but apparently did not consider this due to Diplodia seedling blight.

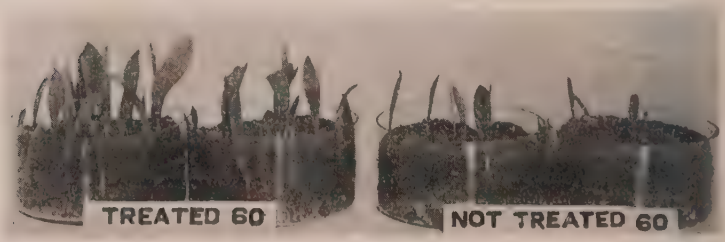

Fig. 5. Plants grown in visible root sand cultures at 15° to 19° C. from Diplodia-infected yellow dent seed corn treated with Merko (left) and from like seed untreated (right). Water was added to the sand each day to bring it to 60 percent of its moisture holding capacity.

CONTROL OF SEEDLING BLIGHTS OF CORN

In connection with the studies already described on Diplodia infection of the corn plant, experiments bearing on the effective control of seedling blight were undertaken.

The control of Diplodia seedling blight involves either killing or inhibiting the organism in infected seed without inducing any injury to the germinating seed when grown under different moisture and temperature conditions. Two typical experiments bearing on the effect of a mercury dust disinfectant applied to the seed, which was afterward germinated at different temperatures and moisture conditions, are described in detail. In the

Fig. 6. Plants grown in visible root sand cultures at 20° to 24° C. from Diplodia-infected yellow dent seed corn, not treated and treated with Merko, showing the effect of the moisture content of the sand. Each day water was added to the sand in each of these crystallizing dishes to bring it to its original moisture content, namely 95 percent (left), 60 percent (center) and 25 percent (right) of its moisture holding capacity.

TABLE VII. SUMMARIZED DATA SHOWING THE EFFECT OF SEED TREATMENT ON DIPLODIA-INFECTED SEED AS INFLUENCED BY TEMPERATURE AND MOISTURE.

Temperature (degrees C.)	Percentage of moisture holding capacity	Treatment	Percent kernels not germinating	Percent seedlings not emerging	Percent seedlings emerged	Percent seedlings free from lesions	Weight green tops in grams	Percent increase in emerged seedlings following treatment
20 - 24	95	None	20.5	22.5	57.0	0.5	11.50	
"	95	Merko*	9.5	1.5	89.0	77.0	92.75	56.1
"	60	None	13.5	30.0	56.5	2.5	9.00	
"	60	Merko	9.0	2.0	89.0	76.0	74.50	57.5
"	25	None	19.0	10.0	71.0	2.0	5.00	
"	25	Merko	12.5	9.5	78.0	40.0	18.50	9.9
15 - 19	95	None	10.0	49.0	41.0	8.0	8.75	
"	95	Merko	7.5	7.0	85.5	79.0	40.50	108.5
"	60	None	12.0	54.0	34.0	7.5	5.75	
"	60	Merko	9.5	9.0	81.5	76.5	34.00	139.7
"	25	None	15.0	46.5	38.5**	12.0	3.90	
"	25	Merko	12.5	20.5	67.0**	56.0	6.75	74.0

* A seed corn dust fungicide, containing three and one-half percent metallic mercury from the Corona Chemical Division of the Pittsburg Plate Glass Co., Milwaukee, Wis.
**Most of these seedlings were just starting to break thru the surface of the sand.

first trial, 25 kernels were used for each culture, and eight cultures were used in each group. Each group in the temperature

Fig. 7. Corn plants grown in visible root sand cultures at 15° to 19° C. from Diplodia-infected yellow dent seed corn, not treated and treated with Merko, showing the effect of the moisture content of the sand. Each day water was added to the sand in each of these crystallizing dishes to bring it to its original moisture content, namely, 95 percent (left), 60 percent (center) and 25 percent (right) of its moisture holding capacity.

and moisture experiment was matched by a similar group, except that the seed was treated. By this arrangement, there was an untreated and a treated group under two temperature conditions (15°-19° C. and 20°-24° C.) and three moisture conditions (25, 60, and 95 percent of moisture holding capacity). The data were recorded two weeks after planting and are summarized in table VII (also see figs. 5 to 9, inclusive).

The data in table VII show that in all instances seed treatment increased the germination percentage. Seed treatment also increased the percentage of seedlings that emerged. This increase was very significant, except under high temperature and low

Fig. 8. Dent corn plants grown in visible root sand cultures at 15° to 19° C. from Diplodia-infected yellow dent seed corn not treated and treated with Merko. Moisture was kept at 60 percent of the water holding capacity of the sand. (Same plants as in fig. 5.)

moisture content. At the high temperature and the low moisture content, both the treated and untreated Diplodia-infected seed showed high percentages of emergence, which indicates that seed treatment increased the stand comparatively little (9.9 percent). However, at the low temperature and the low moisture content, there was a significant increase (74.0 percent) in emergence from treated seed due to the low percentage stand from the untreated seed. The increase in stand of the treated over the untreated seed varied from 9.9 percent in the warmer dry soil to 139.7 percent in the moderately wet cooler soil. These data show that the most serious seedling blight from Diplodia-infected seed takes place at the lower temperature (15°-19° C.). This does not mean that the organism is less active at the higher tempera-

Fig. 9. Root development of plants grown in visible root sand cultures at 15° to 19° C. from Diplodia-infected yellow dent seed corn untreated, A and C, and like seed treated with Merko, B and D. Moisture of A and B was kept at 95 percent and C and D at 60 percent of the water holding capacity of the sand.

TABLE VIII. EFFECT OF SOIL TEMPERATURE ON THE STAND FROM DIPLODIA-INFECTED SEED CORN AS DETERMINED BY THE VISIBLE ROOT SAND CULTURE METHOD IN THE GREENHOUSES. 25 KERNELS PER CULTURE.

Temperature (degrees C)		Number of plants after two weeks in each of 10 cultures										Total plants	Percent increase in number of plants following treatment
22 - 28	No treatment	10	14	18	14	21	14	9	10	16	18	144	
22 - 28	Merko	21	20	22	24	25	21	21	23	21	25	223	54.8
13 - 18	No treatment	4	5	4	5	4	4	6	3	5	4	44	
13 - 18	Merko	21	22	23	17	16	19	22	19	19	13	191	334.1

tures. It may be noted that in general there are fewer plants free from lesions at the higher temperatures than at the lower temperatures. The plants at the higher temperatures grew more rapidly and were not killed before they reached the surface of the sand. Many of the seedlings grown at the lower temperatures were killed before emergence.

In another trial, in which the low temperature was a little lower than in the foregoing test, even more striking differences in stand were obtained as shown in table VIII and figs. 10 and 11.

Table VIII shows that the increase in stand due to treatment was 54.8 percent at the high temperature and 334.1 percent at the low temperature. Again, the large percentage increase was due to the poor stand from the untreated seed at the low temperature and not on account of a better stand from the treated seed at the high temperature.

Fig. 10. Yellow dent corn plants grown in visible root sand cultures at 13° to 18° C. from Diplodia-infected seed not treated (A) and treated (B) with a mercury dust, Merko.

The foregoing data present additional evidence contrary to the conclusions of Clayton (2) and Durrell (3) that seedling blight is not an important factor at cool temperatures, when Diplodia-infected seed is used.

It seems clear also that the fungus does kill certain kernels while they are still on the cob, and that other slightly infected kernels seem to be damaged little if the progress of the organism is checked as it apparently is by seed treatment. Furthermore, these investigations indicate that it is the rotting of the mesocotyl which is the important consideration rather than competition for food on the part of the host and parasite that determines the ex-

Fig. 11. Plants from untreated (A) and Merko treated (B) Diplodia-infected yellow dent seed corn. (Plants taken from cultures shown in fig. 10.)

tent of the injury. The seedlings died whenever rotting occurred to the extent that it cut off the food supply from the kernels before the crown roots were established.

Field Experiments Using Dust Disinfectants for the Control of Seedling Blights of Corn.

After many of the apparently less valuable treatment materials had been eliminated by means of the visible root sand culture method, field experiments were conducted to test the comparative merits of the more valuable ones. Tests were made upon diseased seed of different kinds and also upon nearly disease-free seed and farmers' seed (a composite sample such as is generally planted by the farmer). The diseased seed was used to determine the disinfecting value of the treatment, the nearly disease-free seed to measure any possible injurious effect of the treatment and the farmers' seed to determine to some extent the value of corn seed treatment for the practical grower.

All composites of nearly disease-free, Gibberella-infected and Diplodia-infected seed were obtained according to the method described by Holbert, Burlison, et al. (6). For the Basisporium composite, only visibly infected kernels were used. Only Diplodia-infected seed and nearly disease-free seed were used in 1926.

In all the experiments, the kernels were carefully counted and planted by hand. In most of the plots, two kernels were planted per hill, and the hills were spaced 40 inches apart each way.

Experiments in 1926

As was stated earlier, many of the preliminary field experiments conducted previous to 1926 indicated that the common disinfectants did not show as much promise as the organic mercury compounds. Also, dust treatments rather than liquid treatments seemed more practical from the standpoint of saving time and labor in application.

For these reasons only three liquid disinfectants, two of which were organic mercuries, and six dust disinfectants were included in trials in 1926. Diplodia-infected seed was used in one plot to determine the efficiency of the fungicides in controlling this organism. Nearly disease-free seed was used in the other plot to measure any injurious effect due to treatment. The plots were planted on May 17 and stand counts were taken when the plants were about 4 inches in height.

The field stands from nearly disease-free seed averaged about 90 percent and were not affected significantly by any of the treatments. All treatments significantly increased the stands from Diplodia-infected seed, approximately from 65 to 80 percent.

Yield data are presented in tables IX and X.

In table IX, it may be noted that there was no significant in-

TABLE IX. YIELD DATA ON EFFECT OF TREATMENT OF NEARLY DISEASE-FREE SEED CORN, USING SIX DUST AND THREE LIQUID FUNGICIDES; 10 REPLICATIONS OF 30 HILLS EACH.

Fungicide	Acre Yield		Increase following treatment		Odds
	Untreated bu.	Treated bu.	bu.	pct.	
S.F.A. 225* (dust)	59.9	61.3	1.4	2.3	3 : 1
Semesan 13**ug (dust)	59.9	61.6	1.7	2.8	4 : 1
Semesan Jr.** (dust)	59.9	59.9	0.0	0.0	
Bayer Dust**** (dust)	60.2	59.8	−0.4	−0.7	1 : 1
Dupont 12 Bel.*** (dust)	60.2	60.1	−0.1	−0.2	1 : 1
Abavit B****** (dust)	60.2	60.4	0.2	0.3	1 : 1
Mercuric chloride 1-3000-1½ hr. (soak)	60.5	58.4	−2.1	−3.5	4 : 1
Uspulun***** 1-200 1½ hr. (soak)	60.5	59.5	−1.0	−1.7	2 : 1
Semesan** 1-200 1½ hr. (soak)	60.5	60.6	0.1	0.2	0.4 : 1

* A mercury cresol-sodium cyanide compound containing about 8 percent toxic reagent from Saccharin-Fabrik Aktiengesellschaft, voem Gahlberg List Magdeburg, Germany.
** Chlorophenol mercury compounds containing about 20, 10 and 30 percent toxic reagent, respectively, from E.I. du Pont de Nemours & Co. (Inc), Wilmington, Del.
*** A mercurated nitrophenol compound containing about 10 percent toxic reagent from E.I. du Pont de Nemours & Co. (Inc.), Wilmington, Del.
**** A mercurated nitrophenol compound containing four percent toxic reagent from the Bayer Company (Inc.), New York, N.Y.
***** A chlorophenol mercury compound containing about 30 percent toxic reagent from the Bayer Company (Inc.), New York, N.Y.
****** A dust disinfectant containing mercury from Chemische Fabrik Ludwig Meyer, Mainz, Germany.

crease or decrease in yield from any of the treatments of nearly disease-free seed. The mercuric chloride soaked seed showed the largest decrease in yield, 3.5 percent, but the odds were only 4 to 1.

Yield data presented in table X show that the increases following the treatment of Diplodia-infected seed were significant in every case. Increases ranged from 6.8 bushels to 13.9 bushels per acre. The best dust treatments gave practically as high yields as the best liquid treatments. In comparison with data presented in table IX, it may be noted that the untreated nearly

TABLE X. YIELD DATA ON EFFECT OF TREATMENT ON DIPLODIA-INFECTED SEED CORN, USING 6 DUST AND 3 LIQUID FUNGICIDES, 10 REPLICATIONS OF 30 HILLS EACH.

Fungicide	Acre Yield		Increase following treatment		Odds
	Untreated	Treated			
	bu.	bu.	bu.	pct.	
S.F.A. 225 (dust)	41.4	52.2	10.8	26.1	9999 : 1
Semesan 13 ug. (dust)	41.4	50.7	9.3	22.5	1666 : 1
Semesan Jr. (dust)	41.4	52.2	10.8	26.1	4999 : 1
Bayer Dust (dust)	40.9	51.6	10.7	26.2	9999 : 1
Dupont 12 Bel. (dust)	40.9	53.4	12.5	30.6	9999 : 1
Abavit B (dust)	40.9	47.7	6.8	16.6	163 : 1
Mercuric chloride 1-3000-1½ hr. (soak)	40.6	49.3	8.7	21.4	666 : 1
Uspulun 1-200-1½ hr. (soak)	40.6	54.5	13.9	34.2	9999 : 1
Semesan 1-200-1½ hr. (soak)	40.6	52.2	11.6	28.6	3332 : 1

disease-free seed yielded about 60 bushels per acre while the untreated Diplodia-infested seed yielded about 41 bushels per acre a difference of about 19 bushels. However, the data in table X show that the best treatment brought the yield from Diplodia-infected seed to over 54 bushels per acre, which was only about six bushels less than the yield from nearly disease-free seed in the adjoining plot. It should be remembered that the diseased seed used in these experiments was much more heavily infected than that used by farmers in general.

Experiments in 1927

Because of the fact that in previous trials dust treatments gave as good results as liquid treatments and did not have many of the disadvantages of the liquids, only dusts were used in the field experiments in 1927. In order to arrive at the actual value of these treatments, many different kinds of seed corn were treated. On the experiment station plots at Ames, Iowa, three strains (A, B, and C) of nearly disease-free seed, three strains (A, B, and C) of Diplodia-infected seed, one strain (D) of Basisporium-infected seed and one strain (E) of Gibberella-infected seed were used.

Each strain of each kind of seed was planted in a separate plot. All figures given are on 10 replications of 30 hills each. The plots were planted May 13 and 14, 1927. Yield data are given in tables XI to XIV.

The yields from the different lots of the same strains of corn given in tables XI and XIV cannot be directly compared since there was considerable variation in the fertility of the soil upon which these different plots were grown. Within each plot the soil variations were not great and there were 10 replications of each treatment and check.

Data in table XI show that the yields from Diplodia-infected seed were increased in every instance, and that the increase varied from 21.3 to 30.2 percent. In all cases the odds were very significant.

On seed infected with *Basisporium gallarum,* data on which are

TABLE XI. DATA ON YIELDS FROM DIPLODIA-INFECTED SEED CORN, NOT TREATED AND TREATED WITH DUST FUNGICIDES; 10 REPICATIONS OF 30 HILLS EACH.

Strain of corn	Fungicide	Acre yield in bu.		Increase following treatment		Odds
		Untreated	Treated	Bu.	Percent	
A	Merko	34.9	42.8	7.9	22.6	4999 : 1
B	Merko	29.1	37.9	8.8	30.2	9999 : 1
B	Bayer Dust	29.1	35.3	6.2	21.3	262 : 1
C	Merko	28.3	36.6	8.3	29.3	4999 : 1
C	Bayer Dust	28.9	37.0	8.1	28.0	2399 : 1
C	Semesan Jr.	28.9	37.3	8.4	29.1	9999 : 1

TABLE XII. DATA ON YIELDS FROM BASISPORIUM-INFECTED SEED CORN, NOT TREATED AND TREATED WITH DUST FUNGICIDES, 10 REPLICATIONS OF 30 HILLS EACH.

Strain of corn	Treatment	Acre yield in bu.	Increase following treatment		Odds
			Bu.	Percent	
D	None	26.5			
D	Merko	30.9	4.4	16.6	416 : 1
D	Bayer Dust	31.7	5.2	19.6	9999 : 1
D	Semesan Jr.	30.6	4.1	15.5	1666 : 1

given in table XII, significant increases in yield were obtained following the use of each of the three commercial dusts, namely, Merko, Bayer Dust and Semesan Jr. The increases varied from 15.5 to 19.6 percent.

Data presented in table XIII show that the increases in yields following treatment of seed infected with *Gibberella saubinetti* were relatively small in comparison with the increases of the other composites.

The increase following the use of Merko was 8.6 percent, while that following the use of Bayer Dust was 3.5 percent. The odds in the case of the Merko treated seed were barely significant, while those in the case of the Bayer Dust treated seed were not significant. This composite contained only a small percentage of kernels infected with *Gibberella saubinetti*, and therefore this experiment merely indicates that it is possible to increase yields from Gibberella-infected seed by treatment of the seed.

In table XIV, it may be noted that with two of the three nearly disease-free strains seed treatments gave no significant increases or decreases. However the yield from the third strain was increased 7.6 bu. per acre or 19.8 percent following treatment with Merko, the only treatment used.

Additional data on treatment of nearly disease-free seed were obtained thru the State Corn Yield Test in 1927.* In each of four districts of the state, namely two, five, seven and ten, four dust treatments and a check were used on a mixed lot and also on a selected lot of seed. Two of these dust treatments were trial dusts, and only the results with the two commercial dusts,

TABLE XIII. DATA ON YIELDS FROM GIBBERELLA-INFECTED SEED CORN, NOT TREATED AND TREATED WITH DUST FUNGICIDES' 10 REPLICATIONS OF 30 HILLS EACH.

Strain of corn	Treatment	Acre yield in bu.	Increase following treatment		Odds
			Bu.	Percent	
E	None	37.2			
E	Merko	40.4	3.2	8.6	31 : 1
E	Bayer Dust	38.5	1.3	3.5	7 : 1

TABLE XIV. DATA ON YIELDS FROM NEARLY DISEASE-FREE SEED CORN, NOT TREATED AND TREATED WITH DUST FUNGICIDES; 10 REPLICATIONS OF 30 HILLS EACH.

Strain of corn	Fungicide	Acre yield in bu.		Increase following treatment		Odds
		Untreated	Treated	Bu.	Percent	
A	Merko	38.4	46.0	7.6	19.8	132 : 1
B	Merko	37.1	36.1	−1.0	−2.7	2 : 1
B	Bayer Dust	37.1	36.3	−0.8	−2.1	3 : 1
C	Merko	31.3	32.1	0.8	2.6	3 : 1
C	Bayer Dust	32.1	33.2	1.1	3.4	7 : 1
C	Semesan Jr.	32.1	31.9	−0.2	−0.6	1 : 1

Merko and Bayer Dust, are given here. The mixed lot used in each district was made with seed from 12 different farmers in the county in which the test field was located. The selected lot of seed used in each district was a carefully selected farmers' entry, which was divided into five lots, four of which were treated and one left untreated as a check. The mixed lots were handled in a like manner. A germination test of each of these lots of seed showed practically no disease, and therefore they may be regarded as nearly disease-free. It was hoped that the composite lots would represent average farmers' seed, but apparently most of this seed had been carefully selected. All entries were planted in 25 hill rows, replicated 10 times. Yield data were taken by those in charge of the tests. Results are given in table XV.

Table XV shows that of the four composite lots of seed, each treatment gave two increases and two decreases in yield. Both treatments gave an increase in yield from the selected seed in every instance. The increases following the treatment of the seed with Bayer Dust ranged from 0.9 to 4.6 bu., while the increases following the use of Merko ranged from 2.6 to 8.0 bu. per acre. These results indicate that certain strains of nearly disease-free corn are benefited more than others by seed treatment.

*Hughes, H. D., Robinson, Joe L., and Bryan, A. A. Bul. 265, Iowa Agr. Exp. 1929.

TABLE XV. YIELD DATA ON THE EFFECT OF TREATING MIXED LOTS OF SEED CORN AND SELECTED SEED CORN WITH MERKO AND BAYER DUST, SECURED IN 1927 FROM ENTRIES IN THE IOWA STATE CORN YIELD TEST IN WHICH PLOTS OF 25 HILLS ARE REPLICATED 10 TIMES.

District no. and location in Iowa.	Acre yields in bu.		Increase in yield following treatment in bu.			
	Mixed seed	Selected seed	Mixed seed		Selected seed	
			Merko	Bayer Dust	Merko	Bayer Dust
2, North	31.6	36.7	1.7	2.0	3.2	0.9
5, North Central	47.2	40.7	3.9	−3.2	2.6	1.8
7, South Central	52.7	47.2	−2.8	−1.9	3.6	4.6
10, South	46.5	51.9	−1.2	0.2	8.0	1.0
Average			0.4	−0.7	4.4	2.1

The results obtained are in accord with those of Reddy, Holbert and Erwin (11) and Reddy and Holbert (10) on sweet corn and with those of Holbert, Reddy and Koehler (7) and Melhus et al. (9) on dent corn. Kiesselbach (8), on the other hand, obtained no beneficial results from treatment of seed corn. Most of his experiments were conducted under very dry conditions, which may have contributed to his failure to obtain increases in yield following treatment.

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